Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

The
Journal of Infectious
Diseases

PUBLISHED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. 9 *September 1911* No. 2

ON THE LOCAL PRODUCTION OF ANTIBODIES.*

LUDVIG HEKTOEN.
(From the Memorial Institute for Infectious Diseases, Chicago, Ill.)

Whether the power to produce antibodies is widely distributed among the cells of the body or whether it is limited more or less strictly to certain cells or tissues has not been determined definitely. Under the guidance of Ehrlich's lateral chain hypothesis which, broadly speaking, assumes that any cell that takes up or binds antigen can produce the corresponding antibody or antibodies, certain experiments have been made for the purpose of determining whether the tissues at the site of the introduction of antigen respond by a local production of antibody. The tissues in question are the conjunctiva and those of the anterior chamber of the eye; the tissues of the pleura and peritoneum; and the subcutaneous tissues.

THE CONJUNCTIVA AND THE ANTERIOR CHAMBER.

Römer[1] reached the conclusion that on immunization of rabbits with abrin by way of the conjunctiva, the conjunctiva itself furnishes part of the resulting antitoxin at the same time as that abrin which is absorbed into the general circulation calls forth antitoxin

---

* Received for publication June 10, 1911.

[1] "Experimentelle Untersuchungen über Abrin (Jequiritol)-Immunität als Grundlagen einer rationellen Jequirity-Therapie," *Arch. f. Opthalmol.*, 1901, 52, p. 72.

production in the spleen and marrow. It may be pointed out, however, that at the time when Römer made his tests which showed that the spleen, the marrow, and the conjunctival tissue (of the eye exposed to abrin) contained antitoxin the blood was also antitoxic. One of the rabbits had been under treatment for six weeks, the other for three, and during this time the conjunctiva in question was repeatedly subjected to the action of increasing quantities of abrin. It consequently is not excluded that the antitoxic action of the conjunctival tissue may not have resulted from the passage of antitoxin from the blood and lymph into the conjunctiva. While it is true that the conjunctiva of the untreated eye was devoid of antitoxic power, the repeated attacks of inflammation in the eye exposed to abrin would have rendered much more easy the passage of antitoxin into this conjunctiva. In active as well as passive immunity of rabbits to sheep's blood, Miyashita[1] finds that the lytic amboceptor passes into the normal corneal tissue. This is not surprising in view of the apparently constant presence of antibodies in the lymph when they occur in the blood, the concentration being not far below that of the blood.[2] Here may be mentioned also the observation of Flexner and Clark[3] to the effect that in experimental poliomyelitis of monkeys the specific immune substances, elaborated outside of the nervous tissues, in the earlier stages of the attack escape into the cerebrospinal fluid; later, however, as the vessels return to normal, this passage ceases. Hence the apparent production of antitoxin by the treated conjunctiva as well as its immunity to abrin in Römer's experiments may be explained as a result of the general immunity by virtue of which antitoxin in the blood and lymph was deposited in the conjunctival tissue.

V. Dungern[4] found that so long as the iris was not injured in any way no specific precipitin would appear in the aqueous humor in rabbits immunized with maja-plasma by subcutaneous injection.

---

[1] "Die Immunitätsverhältnisse der Hornhaut," *Ztschr. f. Immunitätsf.*, 1911, 9, p. 541.

[2] Becht and Greer, "A Study of the Concentration of Antibodies in the Blood of Normal and Immune Animals," *Jour. Infect. Dis.*, 1910, 7, p. 127; Hektoen and Carlson, "On the Distribution of Antibodies and Their Formation by the Blood," *ibid.*, p. 319.

[3] "Experimental Poliomyelitis in Monkeys," *Jour. Am. Med. Assn.*, 1911, 56, p. 585.

[4] *Die Antikörper*, Jena, 1902.

In a number of experiments he first withdrew the contents of the anterior chamber and filled it again with crab-plasma (*Maja-squinado*) diluted four times with water; in some instances the experiment failed for various reasons, notably infection, and in two such cases precipitin appeared in the blood and not in the fluid in the chamber; in two other instances no precipitin was demonstrated either in the blood or the humor; but in one animal the result seemed to indicate that precipitin was formed by the cells about the anterior chamber. In this case a few drops were injected into the chamber of the right eye and eight days later the aqueous humor of this eye gave precipitate while the serum of the blood and the aqueous humor of the left eye gave none at all. The same result was obtained on the following day, and on the day after that the blood serum also caused a precipitate; for a long time thereafter precipitin was present in the humor of the right eye and in the blood. Here it does seem as if in this case precipitin was formed by the cells about the anterior chamber, but v. Dungern's conclusion from this single result that all kinds of cells may form antibodies and his suggestion that in this case the precipitin in the blood also was formed in the anterior chamber are open to doubt.

Proceeding along the same lines I have made some experiments involving the anterior chamber of the eye in dogs. First the aqueous humor was removed by means of syringe and needle, special care being taken not to puncture the iris or any other vascular structure; the anterior chamber of one of the eyes was then filled with a thick and sterile suspension of rat or goat corpuscles, that of the other eye with sterile salt solution. The fluids of the anterior chambers, were removed by puncture from time to time and tested as to their content in specific antibody at the same time as the antibody content of the serum of the blood was determined. So far as the normal aqueous humor is concerned it contains practically no antibody for rat or goat corpuscles; sometimes a trace of opsonin for rat corpuscles is found. In no case did the fluid of the anterior chambers in these experiments give any evidence of the presence of antibody until after newly formed antibodies appeared in the blood, and the amount of antibody in the humor of the injected eye was always much less than that in the blood serum.

In some cases the fluid in the anterior chamber of the control eye also contained antibody but usually not until after the second puncture. In all cases the amounts of antibody in the anterior chambers were greater the more marked the inflammatory reaction.

The general results here outlined are illustrated in the following tables:

TABLE 1.

SHOWING THE CONCENTRATION OF SPECIFIC AGGLUTININ AND OPSONIN IN THE FLUIDS OF THE ANTERIOR CHAMBERS AND OF THE BLOOD SERUM OF DOGS INJECTED WITH RAT CORPUSCLES IN THE ANTERIOR CHAMBER OF THE RIGHT EYE.

DAYS AFTER INJECTION	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		
	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum
0	0	0	24	0	0	48	0	0	48
2	..	..	24	..	..	96	..	..	..
3	0	0	48	0	0	96	..	..	..
4	0	0	196	0	0	96	0	0	192
5	..	..	..	12	6	96	..	..	..
6	6	0	96	..	..	..	..	..	..
7	..	..	..	12	6	384	..	..	..
8	..	..	..	6	0	384	..	..	..

The figures give the highest dilution in which the fluids in question gave distinct agglutination and opsonification; 0 means no effect in a dilution of 1 to 6.

TABLE 2.

SHOWING THE CONCENTRATION OF SPECIFIC LYSIN IN THE FLUIDS OF THE ANTERIOR CHAMBERS AND OF THE BLOOD SERUM OF DOGS INJECTED WITH GOAT CORPUSCLES IN THE ANTERIOR CHAMBER OF THE RIGHT EYE.

DAYS AFTER INJECTION	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		
	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum	Right Eye	Left Eye	Blood Serum
0	0	0	96	0	0	96	0	0	96
2	..	...	...	...	...	...	6	0	192
3	...	...	...	...	...	...	6	6	192
4	192	0	1,536	192	0	384	6	6	384
5	...	...	...	...	...	...	24	12	384
6	...	...	...	384	192	1,536	...	...	...
7	...	...	...	...	...	...	...	...	...
8	...	192	12,288	...	...	...	...	...	...

The figures give the highest dilution in which the fluids in question gave distinct lysis; 0 means no effect in dilution of 1 to 6. The fluids were heated to 58° C. for 30 minutes before tested and fresh guinea-pig serum was used as complement (see p. 107 for quantities).

THE PLEURA AND THE PERITONEUM.

In order to determine whether in rabbits the tissues of the pleura and the peritoneum have the power to produce lysin for typhoid bacilli Wassermann and Citron[1] determined the amount of lysin in the blood serum and serum of peritoneal and pleural exudates after single injections of bacilli intravenously, intraperitoneally, and intrapleurally. The exudates were produced by means of suspensions of aleuronat. The results obtained are interpreted as indicating the possibility of formation of antibodies by the cells of the peritoneum and the pleura because in some cases the serum of the exudate in animals given pleural and peritoneal injections produced lysis of typhoid bacilli in higher dilutions than the blood serum of those animals. This seemed true especially in the case of the pleura.

---

[1] "Ueber die Bildungsstätten der Typhusimmunkörper. Ein Beitrag zur Frage der lokalen Immunität der Gewebe," *Ztschr. d. Hyg. u. Infektionskr.*, 1905, 50, p. 331.

I have made similar experiments on dogs, using rat and goat corpuscles as antigens. To obtain exudate, suspension of aleuronat was injected into the pleural cavity, the resulting fluid being withdrawn 10 to 14 hours later and again in most instances at the end of the next 24 hours (Tables 3 and 4). The clear serum obtained after centrifugation of the exudate was used in the tests. In all cases special care was taken to secure exudate free from blood. In the case of the dogs injected with rat corpuscles as well as in the case of those injected with goat blood the final test was made at the same time so that in each case all the samples of serum were tested with the same rat or goat corpuscles as the case might be. The sera were first heated to 50° C. for 30 minutes. The antigoat sera were reactivated with the same guinea-pig serum in the determination of their lytic powers, 0.0125 c.c. of guinea-pig serum being used in each test, the total quantity of each mixture being 0.6 c.c., of which 0.2 c.c. was five per cent suspension of carefully washed goat corpuscles; the remainder of the mixture was $M/8$ NaCl solution, the necessary quantity of immune serum, and 0.0125 c.c. fresh guinea-pig serum. The mixture for testing the agglutinating and opsonic powers of the antirat sera contained simply 0.2 c.c. five per cent suspension of washed rat corpuscles, the requisite amounts of immune serum, and sufficient salt solution so that each mixture equaled 0.6 c.c. The preliminary tests made immediately after the sera were obtained gave the same results as those given in the tables.

In Table 5 are given results obtained with the sera of dogs injected with goat blood and killed 3, 6, 9, and 12 days after the

TABLE 3.

SHOWING THE AMOUNT OF ANTIBODY (AGGLUTININ) IN THE BLOOD AND IN PLEURAL EXUDATE AFTER THE INJECTION OF RAT CORPUSCLES (1 C.C. 10 PER CENT SUSPENSION PER KILO OF WEIGHT OF DOG) (*a*) INTRAVENOUSLY AND (*b*) INTRAPLEURALLY.

DAYS AFTER INJECTION	INTRAVENOUS INJECTION OF ANTIGEN				INTRAPLEURAL INJECTION OF ANTIGEN			
	Dog I		Dog II		Dog I		Dog II	
	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate
0	96	....	48	....	96	....	48	....
2	24	....	48	....	96	....	48	....
4	96	....	96	....	192	....	48	....
5	768	384	768	....	384	....	192	384
6	1,536	....	1,536	....	768	....	768	....
7	3,072	1,536	3,072	1,536	....	....	1,536	1,536
8	6,144	3,072	6,144	....	3,072	....	3,072	....
9	6,144	3,072	3,072	3,072	3,072	3,072	3,072	3,072
11	6,144	3,072	6,144	6,144	3,072	3,072	1,536	1,536
13	6,144	3,072	6,144	6,144	6,144	3,072	1,536	1,536
15	3,072	....	6,144	....	3,072	1,536	1,536	....
18	3,072	....	6,144	....	3,072	....	3,072	....
20	3,072	3,072	3,072	....	3,072	1,536	3,072	1,536
24	3,072	....	3,072	....	3,072	....	3,072	....
28	Killed	....	3,072	....	3,072	....	3,072	....
33	....	....	3,072	....	Killed	....	1,536	....
38	....	....	3,072	....	....	....	768	....
39	....	....	3,072	1,536	....	....	768	768
45	....	....	3,072	....	....	....	768	....
52	....	....	3,072	....	....	....	192	....
59	....	....	1,536	....	....	....	192	....
66	....	....	768	....	....	....	192	....
73	....	....	768	....	....	....	192	....
80	....	....	768	....	....	....	192	....

The figures give the highest dilution in which the sera were active. Determinations of opsonin at various times gave results in exact harmony with those in the table.

TABLE 4.

SHOWING THE AMOUNT OF ANTIBODY (LYSIN) IN THE BLOOD AND IN THE PLEURAL EXUDATE AFTER THE INJECTION OF GOAT CORPUSCLES (1 C.C. 10 PER CENT SUSPENSION PER KILO OF WEIGHT OF DOG) (*a*) INTRAVENOUSLY AND (*b*) INTRAPLEURALLY.

DAYS AFTER INJECTION	INTRAVENOUS INJECTION OF ANTIGEN				INTRAPLEURAL INJECTION OF ANTIGEN			
	Dog I		Dog II		Dog III		Dog IV	
	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate	Blood Serum	Pleural Exudate
0	24	....	24	....	24	....	24	....
2	24	....	24	....	48	....	24	....
4	1,536	768	1,536	768	768	768	1,536	768
5	24,576	....	6,144	3,072	3,072	768	12,288	6,144
7	49,152	....	49,152	....	6,144	....	24,576	....
9	49,152	49,152	24,576	12,288	12,288	3,072	24,576	12,288
10	49,152	49,152	24,576	24,576	6,144	3,072	49,152	12,288
12	49,152	....	24,576	....	6,144	....	24,576	....
14	24,576	24,576	12,288	12,288	3,072	1,536	12,288	....
15	49,152	24,576	12,288	6,144	6,144	1,536	....	....
17	24,576	....	6,144	....	6,144	....	....	....
19	12,288	6,144	6,144	6,144	6,144	1,536	....	....
21	12,288	....	6,144	....	3,072	....	....	....
24	12,288	....	6,144	....	3,072	....	....	....
28	6,144	....	6,144	....	1,536	....	....	....

The figures give the highest dilution in which the sera were active.

injection, aleuronat suspension being injected into the pleural and peritoneal cavities about four hours before the animals were killed. In this case peritoneal exudate was also tested. It should be noted that in the experiments represented in this table the exudates were obtained much earlier than in those represented in Tables 3 and 4, and furthermore, that in the animals thus killed the effects of the injection of aleuronat suspension on the leukocytic content of the blood and hence on the antibody-forming functions of the hematopoietic organs necessarily would be much less than in the cases of the animals that lived on after receiving the injections of aleuronat.

TABLE 5.

SHOWING CONCENTRATION OF ANTIBODIES IN BLOOD SERUM AND EXUDATE SERUM AFTER INTRAPLEURAL AND INTRAVENOUS INJECTIONS OF GOAT BLOOD IN DOGS (1 C.C. 10 PER CENT SUSPENSION OF GOAT BLOOD PER KILO OF DOG).

I. *Intrapleural Injection.*

DAYS AFTER INJECTION	BLOOD SERUM			PLEURAL EXUDATE			PERITONEAL EXUDATE		
	Lysin	Agglutinin	Opsonin	Lysin	Agglutinin	Opsonin	Lysin	Agglutinin	Opsonin
3	192	12	12	96	12	6	...	...	...
6	768	48	96	384	24	48	384	24	48
9	6,144	192	192	768	24	24	...	...	...
12	384	12	48	192	12	24	96	12	12
II. *Intravenous Injection.*									
3	384	24	24	...	...	...	...	...	...
6	24,576	192	384	6,144	96	96	6,144	96	96
9	24,576	192	192	6,144	96	96	...	...	...
12	6,144	96	192	...	...	...	...	...	...

In this experiment the dogs were killed at the end of 3, 6, 9, and 12 days after the injections; the exudates were obtained about four hours after the injection of aleuronat. The opsonin determinations were made with leukocytes from pleural exudates in dogs, and carefully washed in order to free them from serum.

The results of these experiments indicate that in most cases the intrapleural injections of the antigen give less concentration of antibody in the serum of the blood than the intravenous injections. Furthermore, that in no case after intrapleural injections of antigen did the content of antibody in the serum of the pleural exudate exceed that in the serum of the blood; often it is a little less. The relation between the antibody content of the exudate and that of the blood seems to be almost the same whichever way the antigen was introduced.

SUBCUTANEOUS TISSUES.

Wassermann and Citron injected dead typhoid bacilli into the ears of rabbits and ligated the ear for two hours; they determined the lytic power of the blood serum on the tenth day, and then amputated the ear. In one rabbit of several thus treated there was a much greater and more rapid fall after the amputation than in the control animals; and this result they hold points to a local production of antibody by the connective tissue of the ear. Inasmuch as many determinations were not made, so that a comprehensive idea of the course of antibody production in these animals could have been obtained, this result alone cannot well be given any decided significance.

That specific vaccine may cause antibody production in a body that is already infected is referred by Wright[1] and others to induced activity of the tissues at the site of the injection, and that is usually the subcutaneous tissue. This view is voiced very clearly by Russell[2] in his explanation of how typhoid vaccine is beneficial in typhoid fever. He holds that the subcutaneous tissue produces antibodies freely, and that as it is not ordinarily involved in typhoid fever, subcutaneous injections of vaccine in this disease do good by throwing this unused center into action.

Forssman[3] and others have observed that the rate and manner of production of certain antibodies, as shown by the curves obtained with the serum of the blood, seem to depend to some degree on the site of the injection of the antigen. For example, the antitoxin curve obtained after subcutaneous injection of botulismus toxin would differ somewhat (but not radically) from that obtained after intravenous injection of the toxin. Regarding this fact as evidence that distinct cell groups are thrown into action as the injections are made in different places, Forssman suggests that each distinct tissue gives a curve of its own. It is possible, however, that the differences noted, is so far as they are not due to individual variations in the power to produce antibodies, depend simply on differences in the rate and concentration in which the antigen is carried from the places of introduction to the centers where antibodies are produced.

---

[1] *Studies on Immunization*, 1909.

[2] "The Prevention and Treatment of Typhoid Fever with Antityphoid Vaccine," *Bost. Med. and Surg. Jour.*, 1911, 164, p. 1.

[3] "Studien über Antitoxinbildung bei aktiven Immunisierung gegen Botulismus," *Centralbl. f. Bakt.*, I Orig., 1905, 38, p. 463.

If antibodies are produced locally at the site of injection of antigen into the subcutaneous tissue, then it would be reasonable to expect that subcutaneous injections of antigen in many places would give large production of antibodies. Accordingly two dogs were injected with one c.c. per kilo of body weight of 10 per cent suspensions of foreign corpuscles, using goat corpuscles in one case and rat in the other, in 10 different places. In neither case did the resulting antibody curve exceed, or differ materially from, the curves that are produced by a single injection subcutaneously of the same quantity of goat or rat blood. Manifestly much weight should not be placed on this result unless corroborated by the results of other experiments of a like nature but with different dosage of antigen.

Wright[1] suggests that massage after the injection of a vaccine into a limb might give such increase of antibodies in the blood that all doubt as to their local production would be removed. Accordingly rat or goat corpuscles were injected into the muscles of the foreleg in dogs, and when the production of antibodies was well under way the tissues injected were subjected to skilled massage (Dr. Carlström) for 20 minutes, but in no case did the antibody content of the blood carefully tested at hourly or half-hourly intervals after the massage, show any such decided rise as to indicate any production of antibodies at the site of injection of the corpuscles.

Again, if the tissues at the site of the introduction of antigen take an effective part in the production of specific antibodies, then there should be found a distinct difference in the amount of antibody in the blood of animals in which these tissues are removed in the early part of the course of antibody formation as compared with the amount in other animals in which the same tissues are not removed, all other conditions remaining the same as nearly as possible. In order to test this hypothesis experiments were made on dogs injected with goat blood, which in this animal induces, as already indicated, a comparatively liberal production of specific antibodies.

---

[1] *Studies on Immunization*, 1909.

The experiments were made as follows: The same quantity of goat blood in proportion to weight—one c.c. of a 10 per cent

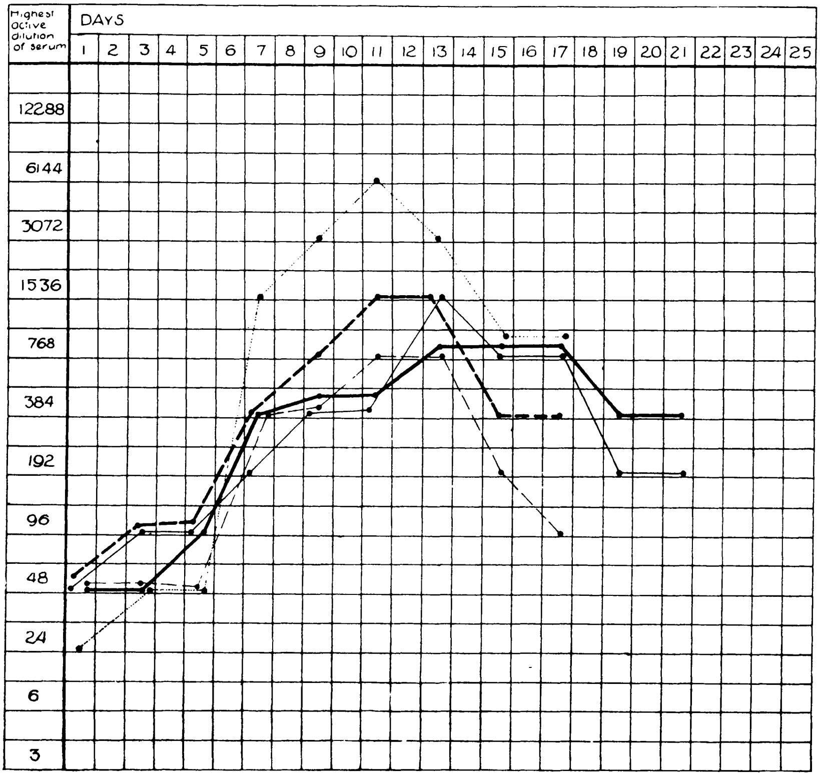

CHART I.—Effect of amputation of foreleg injected with antigen on the production of antibodies.
Heavy lines—Injection of antigen in right foreleg; amputation of right foreleg two days later.
Fine lines—Injection of antigen in right foreleg; amputation of left foreleg two days later.
Dotted lines—Injection of antigen in right foreleg; no amputation.

suspension per kilo of dog—was injected subcutaneously in the lower part of the right foreleg of each of five dogs; two days later the right foreleg was amputated in two of the dogs, and the left in two, while in the case of the fifth dog nothing was done to disturb or modify the formation of antibodies. The subsequent events in the dogs subjected to amputation ran practically the same course; in all there developed some suppuration in the stumps, but in the two represented on Chart 1 by the broken lines this was quite insignificant, and in no case did the general condition appear to be affected. The results so far as the specific lysin in the blood of these dogs is concerned are shown in the chart which shows that there was no striking or definite difference in the amount of lysin in the blood of those dogs whose right forelegs were amputated, as compared with that in the blood of those that lost their left forelegs; in all four the amount is distinctly less than in the fifth dog which was left undisturbed. These results do not indicate that the tissues at the site of the injection of the antigen took any measurable part in the production of antibody.

SUMMARY.

The injection of rat or goat corpuscles in the anterior chamber of the eye of dogs is followed by the appearance of specific antibodies in the blood and usually in the aqueous humor. The concentration of the antibodies is greater in the humor of the injected eye than of the uninjected, but in every case it is much less than in the blood. The antibodies do not appear earlier in the aqueous humor than in the blood.

The injection of rat or goat corpuscles in the pleural cavity in dogs is followed by the appearance of specific antibodies in the blood and in pleural exudates evoked by means of aleuronat. The concentration in the blood is probably a little lower than after intravenous injection of the same amount of antigen. The concentration in the pleural exudates is not higher than in the blood, often it is less. There is no difference in the relation between the antibody content of the blood and of the pleural exudate in dogs receiving the antigen in the pleural cavity in question and in dogs receiving the antigen intravenously.

Massage of the tissues at the site of injection of antigen does not seem to increase the antibody content of the blood in dogs.

In dogs injected subcutaneously over the foreleg with rat or goat corpuscles amputation of the injected leg in the early phases of antibody formation does not result in less antibody in the blood

than in other dogs in which the same tissues are not removed, the other conditions being equal.

The results obtained from the experiments recorded in this article consequently do not point to any local production of specific antibodies in dogs injected with goat or rat corpuscles, at least not so far as concerns the tissues about the anterior chamber of the eye, the tissues of the pleura, and the subcutaneous tissues.